

<p align="center">27 SALICYLATE QUANTITION BY UV/VIS SPECTROPHOTOMETRY</p>	<p align="center">Page 1 of 3</p>
<p align="center">Division of Forensic Science TOXICOLOGY TECHNICAL PROCEDURES MANUAL</p>	<p>Amendment Designator:</p>
	<p>Effective Date: 31-March-2004</p>
<p align="center">27 SALICYLATE QUANTITATION BY UV/VIS SPECTROPHOTOMETRY</p>	
<p>27.1 Summary</p> <p>27.1.1 In the presence of ferric salts, salicylate produces a violet color whose absorbance is a function of salicylate concentration. The intensity of the color in the unknown biological sample is compared with a standard curve of blank blood spiked with salicylate. When run in conjunction with a salicylate-specific immunoassay, this test can be used to confirm the presence of salicylate.</p>	
<p>27.2 Specimen Requirements</p> <p>27.2.1 1 mL blood, biological fluid or tissue homogenate.</p>	
<p>27.3 Reagents and Standards</p> <p>27.3.1 Sodium salicylate</p> <p>27.3.2 Hydrochloric Acid</p> <p>27.3.3 Ferric nitrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$)</p> <p>27.3.4 Mercuric chloride</p> <p>27.3.5 Chloroform</p>	
<p>27.4 Solutions, Internal Standard, Calibrators and Controls</p> <p>27.4.1 0.1 M HCL: Add 3.1 mL concentrated HCl to 100 mL volumetric flask and QS to volume with dH_2O.</p> <p>27.4.2 Trinder's Reagent: dissolve 8.0 g of mercuric chloride in approximately 170 mL of dH_2O by heating. Cool the solution and add 24 mL of 1 M HCl and 5.0 g of ferric nitrate ($\text{Fe}(\text{NO}_3)_3 \cdot 9 \text{H}_2\text{O}$). When the ferric nitrate has dissolved, dilute the solution to 200 mL with dH_2O.</p> <p>27.4.3 2 mg/mL salicylate stock solution. Weigh 232 mg of sodium salicylate and transfer to 100 mL volumetric flask. Dissolve the salt in dH_2O and then QS to volume with dH_2O. Add a few drops of chloroform as a preservative.</p> <p>27.4.4 0.5 mg/mL salicylate blood solution. Pipet 2.5 mL of 2 mg/mL salicylate stock solution into a 10 mL volumetric flask. QS to volume with blank blood.</p> <p>27.4.5 To prepare the calibration curve, pipet the following volumes of the 0.5 mg/mL salicylate blood solution into appropriately labeled 16 x 125 mm screw cap test tubes. Add corresponding volumes of blank blood to obtain the final concentrations listed below.</p>	
<p align="center">Amount of 0.5 mg/mL blood salicylate working solution (mL)</p> <p align="center">0.2 0.4 0.6 0.8 1.0</p>	<p align="center">Amount of blank blood (mL)</p> <p align="center">0.8 0.6 0.4 0.2 0</p> <p align="center">Final concentration of salicylate (mg/L)</p> <p align="center">100 200 300 400 500</p>

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<p>27.4.6 Controls</p> <p>27.4.6.1 Salicylate Control. Control may be from an external source or prepared in house using drugs from different manufacturers, lot numbers or prepared by a chemist different than the individual performing the extraction.</p> <p>27.4.6.2 Negative control. Blood bank blood or equivalent determined not to contain salicylate.</p>	
<p>27.5 Apparatus</p> <p>27.5.1 Test tubes, 16 x 125 mm, round bottom, borosilicate glass with Teflon caps</p> <p>27.5.2 1 cm UV-VIS cuvettes</p> <p>27.5.3 UV-VIS spectrophotometer</p> <p>27.5.4 Centrifuge capable of 2000-3000 rpm</p> <p>27.5.5 Vortex mixer</p>	
<p>27.6 Procedure</p> <p>27.6.1 Label clean 16 x 125 mm screw cap tubes appropriately with calibrators, controls and case samples IDs.</p> <p>27.6.2 Prepare calibrators and controls.</p> <p>27.6.3 Prepare reagent blank with 1 mL dH₂O.</p> <p>27.6.4 Add 1 mL case specimens to the appropriately labeled tubes.</p> <p>27.6.5 Add 5 mL Trinder's reagent to each tube while vortexing. Continue vortexing until the precipitate is finely dispersed.</p> <p>27.6.6 Centrifuge at 2000 rpm for 5 minutes.</p> <p>27.6.7 Transfer supernatants to cuvettes.</p> <p>27.6.8 Read reagent blank at 540 nm.</p> <p>27.6.9 Read each sample at 540 nm.</p>	
<p>27.7 Calculation</p> <p>27.7.1 Plot curve of standard salicylate concentration versus absorbance at 540 nm. Use a linear regression to determine the correlation coefficient and regression line. Calculate case sample salicylate concentrations from standard curve.</p>	
<p>27.8 Quality Control and Reporting</p> <p>27.8.1 Blood blank salicylate concentration should be less than 100 mg/L.</p> <p>27.8.2 Case samples with salicylate concentrations greater than 300 mg/L should be confirmed with a different chemical principal (immunoassay) before being reported.</p>	

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<p>27.9 References</p> <p>27.9.1 Sunshine, I. Methodology for Analytical Toxicology. CRC Press, 1975.</p> <p>27.9.2 Clarke, EGC. Isolation and Identification of Drugs, Vol 1. The Pharmaceutical Press, London, 1969.</p>	